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SCIENTIFIC MEMOIRS

BY

OFFICERS OF THE MEDICAL AND SANITARY DEPARTMENTS

OF THE

GOVERNMENT OF INDIA.

SPECIFICITY OF ANTIVENOMOUS SERA.

(SECOND COMMUNICATION.)

BY

CAPTAIN GEO. LAMB, M.D. (GLASG.)

(Indian Medical Service.)

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SPECIFICITY OF ANTIVENOMOUS SERA.

(SECOND COMMUNICATION.)

IN a previous paper[1] entitled "Specificity of Antivenomous Sera" I drew attention to the great importance and interest of this question both from the practical and from the theoretical standpoint. Further, in this communication I brought forward evidence which pointed to an antivenomous serum being in all probability strictly specific, that is to say, that a serum prepared by injections of a single pure venom is able to neutralise that venom only and no other. This evidence, stated briefly, consisted of the following facts:—

Firstly, the antivenomous serum sent out by Calmette of Lille and prepared with a mixture of venoms, in which mixture cobra venom greatly preponderates, while able to neutralise the general action *in vivo* of cobra venom, has been shown by Martin and Tidswell to have no effect on the venoms of several of the Australian snakes. Further, this serum had no antitoxic effect on the poisons of three of the Indian snakes, namely, Bungarus fasciatus, Vipera Russellii and Echis carinata.

Secondly, the serum of a horse prepared by Tidswell with injections of a pure unmixed venom, namely, the poison of Hoplocephalus curtus, while able to neutralise the poison with which it was prepared, had no effect on the venoms of three other Australian snakes, namely, the brown and black snakes and the death adder. Further, this serum was shown to be quite inoperative against the venoms of three Indian species, namely, Naja tripudians, Bungarus fasciatus and Vipera Russellii.

The observations which led up to these conclusions fell short of a complete study of the question of specificity in several ways.

In the first place, many of the observations were made with a serum, namely, Calmette's serum, prepared with a mixture of snake venoms. Although cobra venom forms the greater part of this mixture, it is *à priori* evident that such a serum is not strictly adapted for the settlement of the question of specificity. This objection also applies to the recent observations published by Rogers,[2] as these observations were also made with Calmette's serum.

In the second place, the two sera were tested against the poisons of only a very few species of snakes.

In the third place, the sera were tested against only the general actions *in vivo* of the several poisons, the experiments being all made on living animals. No attempt was at that time made to test the sera against any particular action of the venoms, such as the action which all venoms exert on the red

blood cells as evidenced by hæmolysis, or the action which they have on the blood plasma which results in an increase or a diminution of its coagulability. Since the date of the first publication I have had the opportunity of greatly extending the observations summarised above in the various directions in which they fell short. These observations form the subject of the present communication.

Firstly.—I have had at my disposal two different antivenomous sera, each prepared with a pure unheated venom, namely, (1) the serum prepared by Tidswell with the poison of Hoplocephalus curtus, and (2) a serum prepared by myself with pure unheated cobra venom. I am greatly indebted to Dr. Tidswell for a further supply of his serum. For the preparation of the cobra venom immune serum the horse was the animal employed. The horse, the serum of which was used in all the experiments which I have now to record, had been treated at the time of the present observations for a period of over two years with gradually increasing doses of pure unheated cobra venom. Beginning with an injection of 0·001 gramme of venom the dose was gradually increased, until at the time of the experiments now described a quantity of 0·7 gramme was being given at each injection without causing any symptoms beyond a considerable local reaction.

Secondly.—I have been able to test these two sera against the poisons of a great many more species of snakes.[3] These may be grouped as follows:—

I.—Colubridæ.

A. Sub-family—Elapinæ.

Genus Naia	1. Naia tripudians (cobra). India.
	2. Naia bungarus (king cobra). India.
Genus Bungarus	3. Bungarus cœruleus (common krait). India.
	4. Bungarus fasciatus (banded krait). India.
Genus Hoplocephalus	5. Hoplocephalus curtus (tiger snake). Australia.

B. Sub-family Hydrophiinæ.

Genus Enhydrina	6. Enhydrina valakadien (common sea snake). India.

II.—Viperidæ.

C. Sub-family—Viperinæ.

Genus Vipera	7. Vipera Russellii (daboia). India.
Genus Echis	8. Echis carinata (phoorsa). India.

D. Sub-family—Crotalinæ.

Genus Trimeresurus	9. Trimeresurus gramineus (green pit viper). India.
Genus Crotalus	10. Crotalus adamanteus (Californian rattlesnake). America.

Thirdly.—I have tested the two antivenomous sera mentioned above not only against the general actions of these venoms *in vivo*, but also, in most cases, against their hæmolytic actions and against their actions on the blood plasma *in vitro*.

It is well to state at the outset that it is not proposed in the present communication to enter in detail into the differences in the physiological actions of these poisons further than is necessary for the purpose in view.

With this introduction we may now pass on to the consideration of the experiments made with the object of ascertaining if either serum had any neutralising effect on the general action *in vivo* of the various poisons. We shall take up the venom of each species *seriatim*. To avoid repetition it would, however, be well to state now the method which in most instances was employed. The test dose of venom, which was always a small multiple of the minimum lethal dose, was mixed *in vitro* with different amounts of serum. The mixtures were allowed to stand at laboratory temperature (about 18°C) for half an hour. They were then injected subcutaneously or intravenously, as the case might be. In all instances rabbits were the animals employed. Attention will be drawn to any exception to this rule of technique.

NAIA TRIPUDIANS (COBRA).—It is acknowledged by practically all observers that cobra venom owes its lethal properties mainly, if not entirely, to its action on the nervous system. In a recent paper [4] along with Dr. Hunter I showed that this action resulted in marked degenerative changes in the nerve cells of all parts of the central nervous system. When a moderate dose is given death is due primarily to a paralysis of respiration, as a result of the action of the poison on the respiratory centres. Cobra venom also causes paralysis of the motor end plates. With a very large dose, especially when the injection is made intravenously, death is probably due to inhibition of the heart brought about by the direct action of the poison on the vagus centres in the medulla.

Its actions on the red blood cells and on the blood plasma, which actions will be considered later on, appear to be secondary, as far as the fatal result is concerned, to the action on the nervous tissues.

I have already shown [5] that the serum prepared with the venom of Hoplocephalus curtus, which serum, for the sake of brevity, will be hereafter designated H. C. V. serum, has absolutely no neutralising power for cobra poison. I have also shown that Calmette's serum has a considerable antitoxic effect against this venom. It, therefore, only remains for us now to consider some experiments which were made with a view of determining the neutralising power of the serum prepared with pure cobra venom (to be known hereafter as C. V. serum), with regard to its corresponding venom.

The test dose of poison used in these experiments was about ten lethal doses. The injections were made subcutaneously. A reference to the protocols (Table I)

will show that 1 c.c. of this serum was able to neutralise at least 1·5 milligrammes of pure unheated cobra venom.

In a previous communication[6] along with Dr. Hanna I put on record the estimation of the antitoxic value of fresh antivenomous serum sent out from Lille for pure unheated cobra venom. As a result of a large series of experiments it was determined that 1 c.c. of this serum was able to neutralise about 0·73 milligramme of poison. I have, therefore, been able to prepare a serum more than double the strength of that prepared by Calmette. Moreover, it may be anticipated that the antitoxic value of this serum will, after the further treatment of the horse with considerably larger doses of poison, become greatly enhanced. Such a result is no doubt due to the fact that a pure and unheated venom was used and not a mixed and heated poison.

NAIA BUNGARUS (KING COBRA).—This species of snake belongs to the same genus as the cobra. Further, as far as can be judged from the observation of symptoms and *post-mortem* appearances, the physiological action of its poison resembles in every way the action of cobra venom. Rogers[7] has recently shown that in this case also death results from respiratory paralysis. We can, therefore, take it that the primary action of the poison is on the nervous tissues. The actions on the blood cells and plasma are also similar to those of cobra venom. Moreover, its toxicity is practically the same as the toxicity of cobra venom. These considerations will show that the series of experiments now to be detailed with this poison and C. V. serum is of the greatest interest and importance.

For the purpose of testing this reaction exactly the same technique was employed as in the experiments detailed in Table I, the test dose of venom used being about ten lethal doses. Under these conditions it will be seen from the protocols (Table II) that even such a large quantity of C. V. serum as 14 c.c. was unable to prevent death. It will, however, be observed that in the case of all the animals which received serum the interval between the injection of the mixture of venom and serum and the death of the animal was somewhat lengthened in comparison with the control animals. It was, however, found that if normal horse's serum was substituted for C. V. serum, death was also delayed, but never for longer than $3\frac{1}{2}$ hours. There is no doubt, therefore, that C. V. serum has a certain hindering effect on the action of king cobra venom *in vivo*. It cannot, however, be said to have a complete neutralising effect even when used in large quantities. Further, it is certain that for practical therapeutic purposes it would be of no value in cases of bites from this snake.

A single experiment with H. C. V. serum and the poison now under consideration served to demonstrate that this serum had absolutely no effect in even delaying death. Such an experiment is detailed in Table III of the protocols.

BUNGARUS CŒRULEUS (COMMON KRAIT).—The symptoms noticed after either intravenous or subcutaneous injection of this venom are indistinguishable

from the symptoms seen in experiments with cobra venom. Rogers [8] has shown that death is due to respiratory paralysis of central origin as in the case of cobra venom intoxication.

The minimum lethal dose for rabbits by intravenous injection was found to be 0·04 milligramme per kilo. The test dose now used to ascertain if either C. V. serum or H. C. V. serum had any antitoxic effect for this venom was ten lethal doses, namely, 0·4 milligramme per kilo.

It will be seen from the protocols (Table IV) that the rabbit which received 4 c.c. of either serum along with the test dose of poison died as quickly as the control animal. It is, therefore, evident that neither C. V. serum nor H. C. V. serum has any neutralising effect for the general action *in vivo* of the venom of Bungarus cœruleus.

BUNGARUS FASCIATUS (BANDED KRAIT).—In a recent publication [9] I gave a detailed account of the physiological actions of the venom of this species. It was there shown that cases of intoxication with this poison can be divided into three classes :—

(*a*) Cases in which rapid death, due to intravascular thrombosis, follows intravenous injection of large quantities of venom.

(*b*) Cases which present acute nervous symptoms and which terminate fatally within two or three days after the injection of the poison. These cases are indistinguishable, as far as symptoms are concerned, from cases of cobra venom intoxication.

(*c*) Cases which run a chronic course and end fatally between the 6th and 12th day after the injection of the poison. Such cases are peculiar to intoxication with this venom and present marked special symptoms. They in no way resemble cases of chronic daboia poisoning. A histological examination of the nervous system in these cases shows a well marked primary degeneration of the cells of the central nervous system.

Further, it was demonstrated in the paper cited that neither Calmette's serum nor H. C. V. serum had any neutralising effect for this poison. We have, therefore, now only to consider an observation made with C. V. serum and Bungarus fasciatus venom.

This observation consisted in testing 4 c.c. of serum against three lethal doses of venom by intravenous injection. A reference to Table V of the protocols will at once show that the animal which received this mixture died after practically the same interval of time as the control rabbit. We can, therefore, conclude that C. V. serum has no antitoxic effect for the action of Bungarus fasciatus *in vivo*.

HOPLOCEPHALUS CURTUS (AUSTRALIAN TIGER SNAKE).—Tidswell [10] has carefully tested the serum which he has prepared with the venom of this species against the corresponding poison and has shown that 0·4 c.c. of serum was

sufficient to neutralise ten lethal doses for the rabbit, namely, 0·59 milligramme. In the same paper he showed that Calmette's serum was quite inactive against the venom of Hoplocephalus curtus. I have forwarded to Dr. Tidswell a supply of the C. V. serum I have prepared, so that he may test it carefully against the poisons of the various species of the poisonous snakes of Australia. The stock of Hoplocephalus poison which I possess is so small that I am unable to undertake any experiments in this direction. As, however, Calmette's serum has no neutralising effect for this venom we may almost conclude from *à priori* reasons that the pure C. V. serum will also be inactive.

ENHYDRINA VALAKADIEN (COMMON SEA SNAKE).—The physiological action of the venom of this and other species of sea snake has been the subject of recent investigation by Rogers.[11] The symptoms produced by this poison may be said to be identical with those seen in cobra venom intoxication. Death is due to respiratory paralysis, the primary action of the poison being on the respiratory centre in the medulla. Rogers also found *post-mortem* paralysis of the motor end plates especially affecting the phrenic nerves. The toxicity of the poison of this species is very much greater than the toxicity of cobra venom. As a preliminary measure to testing the two antivenomous sera the minimum lethal dose of the venom for rabbits by subcutaneous injection was determined. This was found to be about 0·05 milligramme per kilo., a quantity about seven times less than the corresponding minimum lethal dose of cobra venom.

The test dose now employed in both series of experiments was ten lethal doses, namely, 0·5 milligramme per kilo.

The results obtained with C. V. serum are detailed in Table VI of the protocols. From this table it will be seen that 4 c.c. of serum delayed death for a short time; that 10 c.c. of serum had a still more marked effect in this direction, and that 15 c.c. of serum prevented the fatal result. It would appear, therefore, that a serum prepared with unmixed pure cobra venom has a certain neutralising effect for the venom of one of the sea snakes, as far as its action *in vivo* is concerned. This neutralising effect is, however, very slight. For it will be remembered that 1 c.c. of C. V. serum neutralised 1·5 milligrammes of cobra poison. In the case of the venom of Enhydrina valakadien we can calculate that 1 c.c. of the same serum neutralised only 0·031 milligramme, an amount 50 times less than the quantity of cobra venom which was neutralised by the same amount of serum. Allowing even for the greater toxicity of the sea snake venom, it is evident that C.V. serum is far more active for its corresponding venom than for that of Enhydrina valakadien. Further, although the amount of poison injected by this snake is small, estimated by Rogers at about 10 milligrammes, we can say that for therapeutic purposes cobra venom immune serum would be of little or no value in cases of bites from Enhydrina valakadien.

The observations which Rogers has published with reference to the action of

Calmette's serum against the venom of Enhydrina valakadien are somewhat difficult to comprehend. Thus, in one publication,[12] he states, that using only minimum and slightly supra-minimal lethal doses of the poison he found that the serum animals died in just about the same time as the controls. He, therefore, concluded that Calmette's serum is of no use against this poison. Again, in a lecture [13] delivered a month later than this publication, he states, that in a series of experiments in which ten lethal doses were employed Calmette's antivenomous serum was found to have a certain neutralising effect for this poison, as it had also for the venom of the king cobra. It is difficult to reconcile these two statements, and as no details of the experiments performed are given in either instance it is impossible to sift them critically with a view to ascertain where the error lies.

A single experiment was sufficient to show that H. C. V. serum has no antitoxic action for the venom of Enhydrina valakadien. In this experiment the animal which received 4 c.c. of serum along with the test dose, namely, ten lethal doses, died even more quickly than the control animal (*vide* Table VII of protocols).

This experiment completes the observations made *in vivo* with the antivenomous sera and those venoms of the colubridæ which were available. We may sum up the results as follows :—

(1) The serum of a horse immunised with pure cobra venom is strongly antitoxic for the venom used in its preparation : when used in large quantity it has a slight neutralising power for the venom of Enhydrina valakadien, one of the common sea snakes : further, it delays death in cases of intoxication with the venom of the king cobra, a species belonging to the same genus as the cobra: it does not, however, completely neutralise this poison even when used in large quantities. The antitoxic effect against these two venoms is so slight that this serum would be of little or no therapeutic use in cases of bites from these snakes. Finally, this serum contains no antitoxic substances which are active against the venom of either Bungarus cœruleus or Bungarus fasciatus.

(2) The serum of a horse immunised with the pure venom of Hoplocephalus curtus (Australian tiger snake) is strongly antitoxic for its corresponding poison (Tidswell). It has no neutralising power for the poisons of any of the Indian colubrine snakes against which it was tested, namely, Naia tripudians, Naia bungarus, Bungarus cœruleus, and Bungarus fasciatus, nor for the venom of a common sea snake, Enhydrina valakadien.

We may now pass on to the consideration of some observations which were made *in vivo* with the two antivenomous sera and the poisons of four species of Viperidæ, three Indian and one American.

VIPERA RUSSELLII (DABOIA).—I have already shown [14] that, when this venom is injected intravenously into an animal in sufficient quantity, rapid death, the result of intravascular thrombosis, takes place. Further, I have also definitely proved [15] that neither Calmette's serum nor the serum prepared with the venom of

Hoplocephalus curtus has any hindering effect on this action of the poison. A single experiment was now sufficient to show that C. V. serum had likewise no effect of an antitoxic nature against this clotting action of daboia venom *in vivo*, (*vide* protocols, Table VIII).

In one of the communications mentioned above, I pointed out that, if a quantity of daboia venom not sufficient to cause rapid death by intravascular thrombosis be injected, a negative phase of diminished blood coagulability sets in, and that, while the blood is in this condition, the injection of a large quantity of the same poison cannot so increase the coagulability as to cause thrombosis. In a paper[16] recently read before the Royal Society, Rogers has pointed out that, if a preliminary non-lethal dose of venom be given, followed by a larger amount after the phase of diminished coagulability of the blood has set in, quite rapid death could be brought about, in spite of the fact that there was no intravascular thrombosis produced. Further, this observer made a prolonged investigation of this phenomenon and arrived at the conclusion that the primary cause of death in these cases was a complete paralysis of the vaso-motor centre in the medulla. In the case of large animals, such as man, bitten by a daboia he considers that death in most cases will result from this action of the poison. It became, therefore, of great interest to test C. V. serum and H. C. V. serum against this action of daboia venom. The method adopted was as follows: a small non-lethal dose of venom was given intravenously. Half an hour afterwards a mixture containing 4 c.c. of one or other serum and a quantity of poison just capable of causing fairly rapid death by vaso-motor paralysis was injected also intravenously. The constituents of this mixture had been standing in contact for half an hour previous to the injection. A reference to the protocols (Table IX) will show that the two animals which received the sera died in practically the same time as the control. It is evident, therefore, that neither of the sera had any neutralising effect for this action of daboia venom.

ECHIS CARINATA (PHOORSA).—The physiological action of this venom, as far as I have ascertained from a number of preliminary observations, is similar to the action of daboia venom. It is, however, of considerably greater toxicity. Thus, a dose of 0·05 milligramme per kilo. injected intravenously into a rabbit can cause rapid death, the result of intravascular clotting. A smaller amount than 0·05 milligramme per kilo. is non-lethal, but causes a negative phase of diminished coagulability. Further, the action of this poison resembles the action of daboia venom inasmuch as, if after a small non-lethal dose a larger quantity be injected, rapid death ensues, and in these cases no intravascular clotting can be demonstrated. Although I have not investigated this phenomenon, it is probable that we are dealing in this case also with a paralysis of the vaso-motor centre in the medulla. It was, therefore, necessary to test the two sera under consideration against both these actions of the venom.

In the first place, they were tested with two minimum clotting doses, namely,

0·1 milligramme per kilo. of weight. It will be seen from the protocols (Table X) that neither serum had any antitoxic effect as far as this action of the poison was concerned.

In the second place, the sera were tested against the action of the venom on the vaso-motor centre in the same way as has been described in the case of daboia poison. A small preliminary non-lethal dose of poison was injected into the marginal vein of one ear. Half an hour afterwards the mixture of serum (4 c.c.) and venom (0·5 milligramme per kilo., an amount not exceeding 10 lethal doses) was injected into the marginal vein of the other ear. This mixture had been standing *in vitro* at laboratory temperature for half an hour. A reference to the protocols (Table XI) will at once show that neither serum had any effect in hindering this action. We can, therefore, conclude from these two series of observations that neither C. V. serum nor H. C. V. serum has any antitoxic effect for the general action *in vivo* of the venom of Echis carinata.

Trimeresurus gramineus (Green Pit Viper).—I have not been able to obtain sufficient venom from this small species of the rattlesnake family to enable me to work out its physiological action in any detail. It was, however, ascertained that fairly rapid death took place after an intravenous injection into a rabbit of 2 milligrammes per kilo., and that no intravascular clotting could be demonstrated. As this poison is able to coagulate citrate plasma *in vitro*, there is no doubt a larger amount would have caused intravascular thrombosis. A dose of 1 milligramme per kilo. failed to produce any symptoms.

The test dose now used for the purpose of ascertaining if either C. V. serum or H. C. V. serum had any neutralising effect against the general action *in vivo* of this poison was 2 milligrammes per kilo. In each instance 3 c.c. of serum were used. It was found (*vide* Table XII of protocols) that neither serum had any antitoxic effect. The serum animals died after practically the same interval of time as the control. In view of the fact that less than two lethal doses was used as the test dose, we can conclude in general terms that neither serum had any neutralising effect on the general action *in vivo* of the venom of Trimeresurus gramineus.

Crotalus adamanteus (Californian Rattlesnake).—The minimum lethal dose of this venom for a rabbit by intravenous injection was found to be 0·025 milligramme per kilo. An early symptom in all animals which received more than this amount was hæmorrhage from the bowels. The animals which lived long enough became very thin, emaciated and paralysed before death. We have evidently, therefore, in these cases to deal both with a hæmorrhagic action and with an action on the nervous system. In no experiment did I use a sufficient quantity of poison to cause intravascular thrombosis, although this phenomenon is well known to occur when a sufficient amount of venom is injected directly into the circulation.

The amount of venom used to test the sera now under experiment was 0·5

milligramme per kilo. injected intravenously, namely, two lethal doses. In each instance 4 c.c. of serum were tested against this dose. It will be seen from the protocols (Table XIII) that the serum animals died in even a shorter time than the control rabbit. We must, therefore, conclude that both C. V. serum and H. C. V. serum are quite inactive against the general action *in vivo* of the venom of Crotalus adamanteus.

These experiments complete the observations which were made with the two antivenomous sera and those venoms of the Viperidæ which were available, as far as the general actions of the poisons *in vivo* are concerned. We have seen that both sera were tested against four venoms of this description and were found in every instance to be quite inactive. It is unnecessary to comment further on these results.

We may now pass on to the second part of our subject, that is, the consideration of some observations which were made with the object of determining if the antivenomous sera in question had any hindering effect on the hæmolytic action *in vitro* of any of the venoms mentioned above.

It has been well known for many years that snake venoms possess a marked power of breaking up living cells in general and the red blood corpuscles in particular. This hæmolytic action can be demonstrated in most instances both *in vivo* and *in vitro*. Quite recently, however, the work of Flexner and Noguchi [17] in America and of Preston Keyes and Sachs [18] in Ehrlich's laboratory has thrown a flood of light on the intimate mechanism of this particular action of snake poisons. It is not my intention in the present communication to enter into any details as regards this mechanism. It is sufficient for our purpose to mention the fact that it has now been shown that venoms act only as amboceptors, and that they require, in order to bring the red cells to solution, a complement of some kind or other. This complement, as has been shown by Keyes and Sachs in the case of cobra poison, may be either a serum complement or lecithin, which is normally present in the red cells themselves and in serum. I have been able to confirm these observations with cobra poison and have also extended them with the other poisons mentioned above. I found that all these venoms act only as amboceptors requiring complement to bring about the hæmolysis, but that in the case of some of the venoms lecithin, either free or as a constituent of the red cells, cannot take the place of serum complement. These observations, however, will form the subject of another communication. For our present purpose it is sufficient to recognise the facts, that complement is necessary for the hæmolytic action of all venoms, and that lecithin cannot act as complement in the case of some poisons. Therefore, to make the experiments now to be recorded uniform throughout serum complement was used in all cases.

The red cells of the dog, as being very susceptible to hæmolysis by all venoms, were chosen for the present observations. The blood was first gently

defibrinated: the red cells were then washed several times with salt solution (0·85 per cent.), being centrifugalised between each washing. A 5 per cent. suspension of the washed red cells was then made in sterile 0·85 per cent. salt solution. It was found that these manipulations completely removed all serum complement.

As a preliminary measure, it was necessary to determine the minimum complete, or nearly complete, hæmolysing dose of each venom for 1 c.c. of the 5 per cent. red cell suspension, when a fixed amount of serum complement was added. The complement used was that of the dog, and the amount employed was 0·5 c.c. of a two-fold dilution of fresh serum. Each tube thus contained—(1) Varying amounts of venom dissolved in 0·85 per cent. salt solution: (2) 1 c.c. of a 5 per cent. suspension of dog's washed red cells: (3) 0·5 c.c. of a two-fold dilution of dog's fresh serum. The tubes were placed in the incubator (35° C) for one hour and then in the ice chest over night. The results were then recorded. Working in this way the hæmolysing value of each poison was accurately determined, and it was easy to fix the minimum amount of venom which could bring about complete, or almost complete, hæmolysis of a fixed quantity of red cells. This was the amount which was used as the test dose to determine whether either C. V. serum or H. C. V. serum had any hindering effect on the hæmolytic actions of the various poisons. The technique employed was as follows:—

The test dose of poison dissolved in 0·85 per cent. salt solution was mixed with varying amounts of the sera, and the mixtures were allowed to stand at laboratory temperature for at least half an hour. To each tube were then added 1 c.c. of a 5 per cent. suspension of washed dog's red cells and 0·5 c.c. of a two-fold dilution of fresh dog's serum. The preparations were then treated as described above.

In the first place, it was determined that normal horse's serum had no hindering effect on the hæmolysing action of any of the venoms. These experiments need not be detailed.

In the second place, C. V. serum was tested in the manner described above. A reference to the protocols (Table XIV) will show the results which were obtained. It will be seen from this table that C. V. serum had a high neutralising effect for cobra venom itself. The preparation containing 0·04 c.c. of serum showed no hæmolysis, while the tube with 0·02 c.c. showed only a mere trace of laking. We can, therefore, calculate that about 0·02 c.c. of serum was able to neutralise almost completely the hæmolytic action of 0·025 milligramme of cobra venom, the test dose used for these experiments. I have made a number of similar experiments, using larger amounts of poison as a test dose, and have always obtained results corresponding to that just mentioned. Further, it will be seen from this table that C. V. serum prevented to a certain extent the hæmolytic action of the venom of Bungarus cœruleus. A much greater quantity

of serum, however, was required to effect this than in the case of cobra venom. Thus, it required 0·6 c.c. of serum to completely neutralise 0·025 milligramme of this poison, an amount of serum 30 times as much as was necessary for the same quantity of cobra venom. It is to be remembered also that in each instance only a minimum complete hæmolysing amount of venum was used. Finally it will be seen from this table, that C. V. serum had no hindering effect at all on the hæmolysing action of the remaining eight venoms, the tubes containing 1 c.c. of serum showing as complete hæmolysis as the controls.

We may conclude from these observations that the specificity of C. V. serum as regards this particular action of snake venoms is well marked, but not quite absolute. The point of greatest interest and importance is that a serum prepared with cobra venom has absolutely no hindering effect on the hæmolysing action of the venom of the king cobra, a species, as I have mentioned, belonging to the same genus as the cobra.

In the third place, H. C. V. serum was tested against the hæmolytic action of each poison in exactly the same manner as has been described above. It will be seen from the protocols (Table XV) that this serum was not so specific in its action as C. V. serum Thus, it will be noted that it neutralised the venom of Enhydrina valakadien rather better than it neutralised the poison with which it had been prepared. Also, it will be seen that it had a marked hindering effect on the action of cobra venom and on the action of the poison of Echis carinata. It had no hindering effect on the hæmolysing action of the other six poisons against which it was tested.

The results of these two series of observations appear to me to be of the greatest importance. Thus, it is evident that the hæmolysing elements of venoms differ from one another as markedly as do the elements which act on the central nervous system. It cannot even be affirmed that this constituent of the poisons of two species of the same genus are alike. For we have seen that a serum prepared with cobra venom is very active against this poison but has no hindering effect on the hæmolytic action of the poison of the king cobra. In other words, the hæmolysing amboceptors of these two venoms possess different haptophore groups. On the other hand, the red cell receptor of the venom of a colubrine snake may resemble that of the poison of a viperine species. For we have seen that the serum prepared with the venom of Hoplocephalus curtus is active to a certain extent for the poison of Echis carinata. We can, however, conclude that the constituent of any one snake venom which acts as an amboceptor for the red blood corpuscles differs, for the most part, from the similar constituent of any other venom in not possessing exactly the same haptophore groups. There are, as we have seen, exceptions to this rule, but no *à priori* resemblance can be taken for granted in venoms of species belonging to the same genus.

We can now pass on to the third and concluding part of the present inves-

tigation, that is, to the consideration of some observations which were made with the view of ascertaining if either C. V. serum or H. C. V. serum had any hindering effect on the actions of the different poisons on the blood coagulability *in vitro*. It will be convenient in this instance to consider these observations in two groups, namely, (1) the observations made with the venoms which prevent the clotting of citrate plasma which results on the addition of a soluble salt of lime, and (2) the observations made with the venoms which rapidly clot citrate plasma without the addition of any lime salt.

In the first group of poisons are the venoms of the cobra and of the king cobra. In a previous communication [19] I showed that cobra poison had a marked action on the coagulability of the blood plasma, and that this action could be demonstrated *in vitro* with citrate plasma or oxalate plasma. If cobra venom be added in suitable quantity to citrate plasma, the coagulation which normally results from the addition of a suitable amount of a soluble salt of lime is completely inhibited. If the quantity of poison added be too small to completely prevent clotting, coagulation is delayed according to the amount of venom added. In this paper I showed that 1 milligramme of cobra venom was able to prevent completely the clotting of 2 c.c. of citrate plasma, which normally resulted from the addition of a soluble salt of lime.

The poison of the king cobra has a similar action on the blood plasma as that of cobra venom, that is to say, it inhibits or diminishes coagulability. This action can also be demonstrated with citrate or oxalate plasma *in vitro* in the manner I have already described. I found after a series of experiments that 0·8 milligramme of king cobra venom was the smallest amount which could completely inhibit the clotting of 2 c.c. of citrate plasma. The tube containing 0·6 milligramme of poison had a slight clot after standing twenty-four hours.

C. V. serum was now tested against this action of these two venoms. The following was the method employed. The test dose of poison, namely, 1 milligramme in each case, and varying amounts of serum were mixed in small test tubes. The mixtures were allowed to stand at laboratory temperature for half an hour. To each tube there was then added 2 c.c. of citrate plasma. The preparations were left for two hours. Then there was run into each tube a quantity of calcium chloride solution which clotted the control without venom in about 25 minutes. The results were noted at intervals. A reference to the protocols (Table XVI) will show that C. V. serum was able to neutralise cobra venom, as far as this action was concerned, but had little or no effect on king cobra venom. Thus, the experiments show that 0·8 c.c. of serum completely, and 0·6 c.c. nearly, neutralised 1 milligramme of cobra poison, and that even 2 c.c. of serum were not able to neutralise the same amount of king cobra venom. It is true that the tubes containing amounts of serum more than 1·6 c.c. showed a trace of clot after 20 hours. There was evidently

in these tubes an attempt, if I might call it so, at neutralisation, but the venom was still able to act on the plasma in a most decided manner. We can, therefore, conclude that C. V. serum is, as far as this action is concerned, practically specific.

I had not sufficient H. C. V. serum at my disposal to test it against this action of these two venoms on plasma coagulability. As, however, the primary action of the venom of Hoplocephalus curtus on the blood plasma is to bring about a marked increase of coagulability, it is probable that the serum prepared with this poison would be quite inactive against venoms, such as those of the cobra and the king cobra, the primary action of which is to cause a diminution of blood coagulability.

It has long been known that many snake poisons, especially the venoms of the Viperidæ, cause a marked primary increase of blood coagulability which often results in an extensive intravascular thrombosis. In the communication above referred to, I showed that this action of these venoms on the blood plasma could be demonstrated *in vitro*. Thus, it was shown that daboia venom clotted citrate plasma without the addition of any salt of lime, and also that this poison markedly increased the coagulability of oxalate plasma, but did not of itself cause solid clotting. Since this publication I have investigated this phenomenon with the venoms of several other species, and have found that this action of daboia venom on citrate or oxalate plasma is slight in comparison with the similar action of four other poisons, namely, the venoms of Hoplocephalus curtus, of Echis carinata, of Trimeresurus gramineus and of Crotalus adamanteus. If a suitable amount of any of these four venoms be added to either citrate or oxalate plasma, clotting takes place almost instantaneously. Take, for example, the venom of Echis carinata, the most powerful of these four poisons in this respect. Using 2 c.c. of citrate horse plasma (1 per cent.) I found that 0·2 milligramme of venom caused a solid clot in less than one minute, 0·02 milligramme in about ten minutes, 0·002 milligramme in about one hour, and 0·0002 milligramme in at least 20 hours. If these results be compared with the similar observations I have already published with regard to daboia venom, it will be seen that the action of daboia venom on citrate plasma is very much less marked than that of the poison of Echis carinata. Thus, 3 milligrammes of daboia venom caused a solid clot in 2 c.c. of citrate plasma only after 3 hours, and 1 milligramme only after 20 hours. The other three poisons mentioned above are intermediate between the venoms of Echis carinata and Daboia Russellii in the following order of strength :—Crotalus adamanteus, Hoplocephalus curtus and Trimeresurus gramineus.

The two sera under consideration were now tested against this action of this group of poisons. The test dose of each venom used was such an one as caused solid clotting of 2 c.c. of citrate horse plasma in from 5 to 13 minutes. The following was the method employed in all instances. The test dose of

poison was mixed with varying amounts of either serum. The mixtures were allowed to stand at laboratory temperature for half an hour. To each tube were then added 2 c.c. of citrate plasma (1 per cent.). The results were noted at intervals of one minute.

The details of the experiments with C. V. serum are given in Table XVII of the protocols. From this table it will be seen that this serum had no hindering effect at all on this action of any of the four venoms. The details of the experiments with H. C. V. serum are given in Table XVIII of the protocols. A study of this table will make it evident that this serum had a well-marked antagonistic action for the corresponding venom, 0·06 c.c. of serum being able to prevent the clotting caused by 0·1 milligramme of poison. On the other hand, it will be seen that H. C. V. serum had no hindering effect at all on the actions of the other three venoms.

We can, therefore, conclude that, as far as this action of snake poisons is concerned, an antivenomous serum appears to be specific. As, however, the serum prepared with Hoplocephalus curtus venom was not tested against the venoms of any of the other Australian colubrine snakes, which have a similar action on the blood plasma to that of the poison of Hoplocephalus curtus, it is impossible to say that the serum is strictly specific in this action.

Protocols.

The two antivenomous sera, which were used in the observations detailed below, were as follows.

(1) The serum of a horse which had been subjected for a period of two years to repeated doses, gradually increasing in amount, of pure unheated cobra venom. This serum is designated C. V. serum.

(2) The serum prepared by Dr. Tidswell of Sydney.[20] This serum was the serum of a horse which had been subjected to repeated doses, gradually increasing in amount, of the pure unheated venom of Hoplocephalus curtus (Australian tiger snake). This serum is designated H. C. V. serum.

In all the experiments to test the neutralising effect of the two antivenomous sera on the general actions *in vivo* of the several venoms rabbits were the animals employed. The test dose, a small multiple of the minimum lethal dose either by subcutaneous or by intravenous injection, was mixed *in vitro* with different amounts of one or other of the sera. The mixtures before injection were allowed to stand at laboratory temperature for at least half an hour.

TABLE I.—*Experiments to determine the neutralising power of C. V. serum for the general action* in vivo *of pure unheated cobra venom.*

The minimum lethal dose of the sample of venom used for a rabbit by subcutaneous injection was 0·35 milligramme per kilo. The test dose now used was about ten lethal doses.

The following were the results.

Animal.	Weight.	Cobra venom in milligrammes.	C. V. serum.	Result.
Rabbit 1 . .	710 grammes .	2	0·4 c.c. . .	Died in $1\frac{3}{4}$ hours.
„ 2 . .	710 „ .	2	0·6 „ . .	„ $2\frac{3}{4}$ „
„ 3 . .	720 „ .	2	0·75 „ . .	„ 3 „
„ 4 . .	900 „ .	2	1 „ . .	„ 48 „
„ 5 . .	700 „ .	2	1 „ . .	Recovered.
„ 6 . .	770 „ .	2	1·2 „ . .	No symptoms.
„ 7 . .	740 „ .	2	1·25 „ . .	„
„ 8 . .	880 „ .	2	1·25 „ . .	„
„ 9 . .	700 „ .	2	1·5 „ . .	„
„ 10 . .	740 „ .	2	1·75 „ . .	„

The maximum non-lethal dose for a rabbit of 770 grammes in weight (Rabbit No. 6) can be taken at 0·2 milligramme. We can therefore calculate that 1·2 c.c. of serum neutralised at least 2—0·2=1·8 milligrammes, and that 1 c.c. would be able to neutralise 1·5 milligrammes of pure unheated cobra venom.

TABLE II.—*Experiments to ascertain if C. V. serum has any neutralising power for the venom of Naia bungarus (King Cobra).*

The minimum lethal dose of the sample of venom used was about 0·35 milligramme per kilo. by subcutaneous injection. The test dose now used was about en lethal doses.

The following results were obtained.

Animal.	Weight.	N. B. V. in milligrammes.	C. V. serum.	Result.
Rabbit 1 . .	700 grammes .	2	1·6 c.c. .	Died in 5 hours.
„ 2 . .	750 „ .	2	2 „ . .	„ 8 „
„ 3 . .	780 „ .	2	3 „ . .	Found dead after 11 hours.
„ 4 . .	690 „ .	2	4 „ . .	Died in 8 hours.
„ 5 . .	840 „ .	2	5 „ . .	Found dead after 11 hours,
„ 6 . .	610 „ .	2	10 „ . .	Died in 31 hours.
„ 7 . .	700 „ .	2	14 „ . .	„ 31 „
„ 8 (Control) .	750 „ .	2	*Nil* . .	„ 1 hour.

It is evident from these experiments that 14 c.c. of serum failed to neutralise 2—0·21=1·79 milligrammes of venom. Therefore 1 c.c. could not neutralise 0·13 milligramme.

TABLE III.—*Experiments to ascertain if H. C. V. serum has any neutralising power for the venom of Naia bungarus.*

The same sample of venom and the same technique were used as in the previous series of experiments.

The following were the results.

Animal.	Weight.	N. B. V. in milligrammes.	H. C. V. serum.	Normal serum.	Result.
Rabbit 1 . . .	680 grammes .	2	4 c.c. .	*Nil* .	Died in 3½ hours.
„ 2 . . .	780 „ .	2	*Nil* .	4 c.c. .	„ 3½ „
„ 3 (control) .	660 „ .	2	*Nil* .	*Nil* .	„ 1 hour.

It is evident that this serum has no neutralising power for the venom of Naia bungarus.

TABLE IV.—*Experiments to ascertain if either C. V. serum or H. C. V. serum has any neutralising power for the venom of Bungarus cœruleus.*

The test dose of venom used was 10 lethal doses by intravenous injection, namely, 0·4 milligramme per kilo.

The following results were obtained.

Animal.	Weight.	B. C. V. in milligrammes per kilo.	C. V. serum.	H. C. V. serum.	Result.
Rabbit 1 . . .	970 grammes .	0·4	4 c.c. .	*Nil* .	Died in 1 hour.
„ 2 . . .	900 „ .	0·4	*Nil* .	4 c.c. .	„ 47 minutes.
3 (control) .	1,090 „ .	0·4	*Nil* .	*Nil* .	„ 48

It is evident that neither serum has any neutralising power for the venom of Bungarus cœruleus.

TABLE V.—*Experiments to ascertain if C. V. serum has any neutralising power for the venom of Bungarus fasciatus.*

The test dose of venom used was about 3 lethal doses, namely, 2 milligrammes per kilo, by intravenous injection.

The following results were obtained.

Animal.	Weight.	B. F. V. in milligrammes per kilo.	C. V. serum.	Result.
Rabbit 1 . . .	1,050 grammes.	2	4 c.c. . .	Died in 21 hours.
„ 2 (control)	850 „ .	2	*Nil* . . .	„ 26 „

It is evident that this serum has no neutralising power for the venom of Bungarus fasciatus.

TABLE VI.—*Experiments to ascertain if C. V. serum has any neutralising power for the venom of Enhydrina valakadien.*

The test dose of venom used was 10 lethal doses by subcutaneous injection, namely, 0·5 milligramme per kilo.

The following results were obtained.

Animal.	Weight.	E. V. V. in milligrammes per kilo.	C. V. serum.	Result.
Rabbit 1 . . .	650 grammes .	0·5	4 c.c. . .	Died in $4\frac{1}{2}$ hours.
„ 2 . . .	770 „ .	0·5	10 „ . .	„ 20 „
„ 3 . . .	760 „ .	0·5	15 „ .	Recovered.
„ 4 (control) .	610 „ .	0·5	*Nil* . . .	Died in 1 hour.

It would appear from these experiments that 15 c.c. of serum was able to neutralise at least 0·5—0·035 = 0·465 milligramme. It can, therefore, be calculated that 1 c.c. would be able to neutralise 0·031 milligramme.

TABLE VII.—*Experiments to ascertain if H. C. V. serum has any neutralising power for the venom of Enhydrina valakadien.*

The test dose of venom used was 10 lethal doses by subcutaneous injection, namely, 0·5 milligramme per kilo.

The following results were obtained.

Animal.	Weight.	E. V. V. in milligrammes per kilo.	H. C. V. serum.	Result.
Rabbit 1 . . .	630 grammes .	0·5	4 c.c.	Died in 56 minutes.
„ 2 (control) . .	610 „ .	0·5	*Nil*	„ 1 hour.

It is evident that this serum has no neutralising power for the venom of Enhydrina valakadien.

TABLE VIII.—*Experiment to ascertain if C. V. serum has any neutralising effect on the clotting action of daboia venom* in vivo.

The test dose of daboia venom used was about five lethal doses, namely, 0·5 milligramme, by intravenous injection. The following was the result.

Animal.	Weight.	D. V. in milligrammes per kilo.	C. V. Serum.	Result.
Rabbit . . .	880 grammes .	0·5	4 c.c.	Died in 9 minutes.

TABLE IX.—*Experiments to ascertain if either C. V. serum or H. C. V. serum has any neutralising effect on the action of daboia venom on the vaso-motor centre.*

A small non-lethal dose, namely, 0·02 milligramme of venom, was first injected into the marginal vein of one ear. Half an hour after this injection a mixture of 1 milligramme of venom per kilo. and 4 c.c. of serum was injected into the marginal vein of the other ear. This mixture had been standing *in vitro* at laboratory temperature for half an hour. The minimum amount of daboia venom which can cause intravascular clotting in a rabbit by intravenous injection is about 0·1 milligramme per kilo. Any quantity less than this amount is non-lethal. We can take it then that the sera were now tested against not more than ten lethal doses.

The following were the results obtained.

Anim l.	Weight.	Preliminary dose of D. V. in milligrames per kilo.	Test dose of D. V. in milligrammmes per kilo.	C. V. serum.	H. C. V. serum.	Result.
Rabbit 1 . .	960 grammes .	0·02	1	4 c.c.	...	Died in 8 minutes.
„ 2 . .	970 „ .	0·02	1	...	4 c.c.	„ 5 „
„ 3 (control) .	970 „ .	0·02	1	*Nil*	*Nil*	„ 6 „

A careful *post-mortem* examination made immediately after death in each instance failed to reveal any intravascular clotting. The blood collected in a test tube remained permanently unclotted.

TABLE X.—*Experiments to ascertain if either C. V. serum or H. C. V. serum has any neutralising effect on the clotting action* in vivo *of the venom of Echis carinata.*

The test dose of venom used was 0·1 milligramme per kilo., namely, two lethal doses, injected intravenously.

The following were the results.

Animal.	Weight.	E. C. V. in milligrammes per kilo.	C. V. serum.	H. C. V. serum.	Result.
Rabbit 1 . . .	1,220 grammes .	0·1	4 c.c.	...	Died in 1½ minutes.
„ 2 . . .	1,070 „ .	0·1	...	4 c.c.	„ 1½ „
„ 3 (control) .	1,250 „ .	0·1	*Nil.*	*Nil.*	„ 1½ „

TABLE XI.—*Experiments to ascertain if either C. V. serum or H. C. V. serum has any neutralising effect on the action of the venom of Echis carinata on the vaso-motor centre.*

A small non-lethal dose, namely, 0·002 milligramme of venom per kilo., was first injected into the marginal vein of one ear. Half an hour after this injection a mixture of 0·5 milligramme of venom per kilo. and 4 c.c. of serum was injected into the marginal vein of the other ear. This mixture had been standing *in vitro* at laboratory temperature for about half an hour. By previous experiments it had been ascertained that the minimum amount of the venom of Echis carinata which could cause intravascular clotting by intravenous injection into a rabbit was 0·05 milligramme per kilo. Any quantity less than this amount was non-lethal. We can, therefore, take it that the sera were now tested against not more than ten lethal doses.

The following results were obtained.

Animal.	Weight.	Primary dose of E. C. V. in milligrammes per kilo.	Test dose of E. C. V. in milligrammes per kilo.	C. V. serum.	H. C. V. serum.	Result.
Rabbit 1 . .	900 grammes	0·002	0·5	4 c.c.	...	Died in 1 minute.
„ 2 . .	1,050 „	0·002	0·5	...	4 c.c.	„ 2 minutes.
„ 3 (control) .	980 „	0·002	0·5	*Nil.*	*Nil.*	„ 1 minute.

A *post-mortem* examination made immediately after death in each instance failed to reveal any trace of intravascular clotting. The blood collected in a test tube remained permanently unclotted.

TABLE XII.—*Experiments to ascertain if either C. V. serum or H. C. V. serum has any neutralising power for the venom of Trimeresurus gramineus.*

The test dose used, namely, 2 milligrammes per kilo., was less than two lethal doses.

The following were the results.

Animal.	Weight.	T. G. V. in milligrammes per kilo.	C. V. serum.	H. C. V. serum.	Result.
Rabbit 1 . . .	840 grammes .	2	3 c.c. .	...	Died in 9 minutes.
„ 2 . . .	850 „ .	2	...	3 c.c. .	„ 6 „
„ 3 (control) .	810 „ .	2	*Nil.* .	*Nil.* .	„ 3 „

TABLE XIII.—*Experiments to ascertain if either C. V. serum or H. C. V. serum has any neutralising power for the venom of Crotalus adamanteus.*

By previous experiments it was determined that the minimum lethal dose of this venom by intravenous injection was 0·25 milligramme per kilo. of rabbit. This amount killed in $4\frac{1}{2}$ days. The test dose now used was two lethal doses, namely, 0·5 milligramme per kilo.

The following results were obtained.

Animal.	Weight.	C. A. V. in milligrammes per kilo.	C. V. serum.	H. C. V. serum.	Result.
Rabbit 1 . . .	930 grammes .	0·5	4 c.c. .	...	Died in 5 hours.
„ 2 . . .	900 „ .	0·5	...	4 c.c. .	„ 6 „
„ 3 (control) .	900 „ .	0·5	*Nil.* .	*Nil.* .	„ 8 „

TABLE XIV.—*Experiments to ascertain if C. V. serum has any neutralising effect on the hæmolytic action of the venoms of various snakes.*

The venoms used were those already mentioned. The following technique was employed. Dog's blood was defibrinated: the red cells were washed three times with 0·85 per cent. salt solution. A 5 per cent. suspension of the washed red cells was made in 0·85 per cent. sterile salt solution. Of this suspension 1 c.c. was the amount used in each preparation.

The test dose of venom was in each instance the smallest amount which could bring about complete, or nearly complete, hæmolysis of this amount of red cell suspension when a fixed quantity of serum complement was added. This test dose and varying quantities of serum were mixed and allowed to stand in contact for half an hour. To each tube there was then added 1 c.c. of the 5 per cent. suspension of washed red cells and 0·5 c.c. of a two-fold dilution of dog's fresh serum. The preparations were left in the incubator (35°C.) for one hour and

then placed in the ice chest for 18 to 20 hours. The following were the results obtained.

The letters at the top of each column are the initial letters of the genus and species of each snake (*vide* p. 2). The test dose of poison in each instance is indicated at the top of each column.

C. V. serum.	N. T. 0·025 mgr.	N. B. 0·025 mgr.	B. C. 0·025 mgr.	B. F. 0·1 mgr	H C 0·025 mgr.	E. V. 0 1 mgr.	V. R. 0·025 mgr.	E. C. 0·1 m.	T. G. 0·025 mgr.	C. A. 0·025 mgr.
1 c.c.	...	Nearly C. H.	No H.	C. H.	C. H.	Nearly C. H.	C. H.	C. H.	C. H.	C. H.
0·8 „	...	Ditto	No H.	C. H.	C. H.	Ditto	C. H.	C. H.	C. H.	C. H.
0·6 „	...	Ditto	No H.	C. H.	C. H.	Ditto	C. H.	C. H.	C. H.	C. H.
0·4 „	...	Ditto	Trace	C. H.	C. H.	Ditto	C. H.	C. H.	C. H.	C H.
0·2 „	...	Ditto	Marked	C. H.	C. H.	Ditto	C. H.	C. H.	C. H.	C. H.
0·1 „	No H.	...	C. H.	...	...	...	...	...	...	...
0 08 „	No H.	...	C. H.	...	...	...	...	...	...	...
0·06 „	No H.	...	C. H.	...	...	...	...	...	...	...
0·04 „	No H.	...	C. H.	...	...	...	...	...	...	...
0·02 „	Mere trace.	...	C. H.	...	...	..	...	...	...	...
0·01 „	C H.	...	C. H.	...	...	...	...	...	...	...
Nil (control)	C. H.	Nearly C. H.	C. H.	C. H.	C. H.	Nearly C. H.	C. H.	C. H.	C H.	C. H.

C. H. = Complete hæmolysis.

TABLE XV.—*Experiments to ascertain if H. C. V. serum has any neutralising effect on the hæmolytic action of the venoms of various snakes*

Exactly the same technique was employed as in the experiments detailed in Table XIV. The following were the results obtained.

H.C.V. serum.	N. T. 0·025 mgr.	N. B. 0·025 mgr.	B. C. 0·025 mgr.	B. F. 0·1 mgr.	H. C. 0·025 mgr.	E. V. 0·1 mgr.	V. R. 0·025 mgr.	E. C. 0·1 m	T. G. 0·025 mgr.	C. A. 0·025 mgr.
1 c.c.	Mere trace.	Marked	C. H.	C. H.	No H.	No H.	C. H.	Mere trace.	C. H.	C. H.
0·8 „	Ditto	Ditto	C. H.	C. H.	Slight	No H.	C. H.	Ditto.	C. H.	C. H.
0·6 „	Trace	Nearly C. H.	C. H.	C. H.	Nearly C. H.	No H.	C. H.	Trace	C. H.	C. H.
0·4 „	Slight	Ditto	C. H.	C. H.	C. H.	Trace	C. H.	Slight	C. H.	C. H.
0·2 „	Marked	Ditto	C. H.	C. H.	C. H.	Slight	C. H.	Nearly C. H.	C. H.	C. H.
0·1 „	Nearly C. H.	...	...	...	...	...	...	.	...	...
0·08 „	C. H.	...	..	...	...	...	...	...	...	...
Nil (control)	C. H.	Nearly C. H.	C. H.	C. H.	C. H.	C. H.	C. H.	C. H.	C. H.	C. H.

C. H. = Complete hæmolysis.

TABLE XVI.—*Experiments to ascertain if C. V. serum can neutralise the action on citrate plasma* in vitro *either of cobra venom or of king cobra venom.*

The technique employed was as follows: a fixed quantity, namely, one milligramme, of each poison, was mixed in small test-tubes with different amounts of serum. The mixtures were allowed to stand at laboratory temperature for half an hour. There were then run into each tube 2 c.c. of citrate horse plasma (1 per cent.). After these mixtures had stood for two hours, 0·4 c.c. of a 2 per cent. solution of calcium chloride was added to each tube. This amount of lime solution clotted the control in about 25 minutes.

The results were noted at intervals as follows.

Citrate plasma 2 c.c.—$CaCl_2$ (2%) : 0·4 c c.

C. V. Serum.	Cobra Venom (1 milligramme.)	King cobra venom (1 milligramme.)
2 c.c. . . .	.....	Mere trace of clot after 20 hours.
1·8 „ . .	...	Ditto.
1·6 „ . . .	.....	Ditto.
1·4 „ . . .	.....	No clot after 20 hours.
1·2 „ . . .	Clotted solid in 25 minutes	Ditto.
1 „ . . .	Ditto	Ditto
0·8 „ . . .	Ditto	... ..
0·6 „ . . .	Clotted solid in 40 minutes	.....
0·4 „ . .	Found clotted after 20 hours	.....
0·2 „ . . .	Trace of clot after 20 hours	.....
0·1 „ . . .	No clot after 20 hours	.....
0·08 „ . .	Ditto	.....
Nil (control) . .	Ditto	No clot after 20 hours.

The tube with lime alone clotted solid in 25 minutes.

From these experiments it is evident that 0·8 c.c. of C. V. serum completely, and 0·6 c.c nearly, neutralised 1 milligramme of cobra venom: also, that 2 c.c. of serum failed to neutralise the same amount of king cobra venom. There was, however, an attempt at neutralisation in the tubes containing the three greatest quantities of serum.

TABLE VII.—*Experiments to ascertain if C. V. serum can neutralise the actions of the venoms of* (1) *Hoplocephalus curtus*, (2) *Echis carinata*, (3) *Trimeresurus gramineus, and* (4) *Crotalus adamanteus on citrate plasma* in vitro.

These four venoms rapidly clot citrate plasma *in vitro* without the addition of any soluble salt of lime. The technique employed to test if C. V. serum had any hindering effect on this action was as follows.

The test dose of each poison, namely, an amount which clotted the control in a few minutes, was mixed with varying amounts of serum. The mixtures were allowed to stand at laboratory temperature for half an hour. Then into each tube there were run 2 c.c. of citrate plasma. A control tube without serum was prepared in each instance.

The following results were obtained.

Amount of Serum.	H. C. V. (0·1 milligr.)	E. C. V. (0·05 milligr.)	T. G. V. (0·3 milligr.)	C. A. V. (0·05 milligr.)
1 c.c. . . .	Clotted in 6 minutes.	Clotted in 5 minutes.	Clotted in 11 minutes	Clotted in 10 minutes.
0·5 „ . . .	Ditto .	Ditto .	Ditto .	Ditto.
0·1 „ . . .	Ditto .	Ditto .	Ditto .	Ditto.
Nil (control) . .	Ditto .	Ditto .	Ditto .	Ditto.

It is evident from these experiments that C. V. serum cannot prevent the clotting action *in vitro* of any of these four venoms.

TABLE XVIII.—*Experiments to ascertain if H. C. V. serum can neutralise the actions of the venoms of* (*1*) *Hoplocephalus curtus*, (*2*) *Echis carinata*, (*3*) *Trimeresurus gramineus and* (*4*) *Crotalus adamanteus on citrate plasma* in vitro.

The same technique was used as was employed in the observations detailed on Table XVII. The following were the results.

Amount of serum.	H. C. V. (0·1 milligr.)	E. C. V. (0·05 milligr.)	T. G. V. (0·3 milligr.)	C. A. V. (0·05 milligr.)
1 c.c. . . .	Liquid after 24 hours.	Clotted in 5 minutes.	Clotted in 13 minutes.	Clotted in 11 minutes.
0·5 „ . . .	Ditto . .	Ditto . .	Ditto . .	Ditto.
0·1 „ . . .	Ditto . .	Ditto . .	Ditto . .	Ditto.
0·08 „ . . .	Ditto . .	...	...	...
0·06 „ . . .	Ditto . .	...	...	...
0·04 „ . . .	Trace of clot after 24 hours.	...	...	...
0·02 „ . . .	Clotted after 24 hours.	...	...	...
0·01 „ . . .	Clotted in 13 minutes.	...	...	...
Nil (Control) . .	Clotted in 6 minutes.	Clotted in 5 minutes.	Clotted in 13 minutes.	Clotted in 11 minutes.

From the above series of experiment it is seen that 0·06 c.c. of H. C. V. serum completely neutralised 0·1 milligramme of the corresponding venom as far as this clotting action *in vitro* is concerned. It is quite inactive against the similar actions of the three other poisons.

Notes and References.

(1) " Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government of India :" 1903, No. 5.

(2) " Proceedings of Royal Society : " Vol. 72, p. 419. " The Lancet : " 6th February 1904, p. 349.

(3) For the venoms of Naia bungarus and Trimeresurus gramineus I am indebted to the Bombay Natural History Society ; for that of Hoplocephalus curtus to Dr. Tidswell of Sydney ; for that of Enhydrina valakadien to Mr Peal, Calcutta ; and for that of Crotalus adamanteus to Prof. S. Flexner of Philadelphia. The other venoms I collected myself.

(4) "The Lancet :" 2nd January 1904, p. 20.

(5) "*Loc. cit.*"

(6) " Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government of India :" 1902, No. 1.

(7) "*Loc. cit.*"

(8) "*Loc. cit.*"

(9) " Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government of India :" 1904, No. 7.

(10) " Australasian Medical Gazette : " 21st April 1902, p. 177.

(11) " Proceedings of the Royal Society : " Vol. 71, p. 481, and Vol 72, p. 306.

(12) " Proceedings of the Royal Society : " Vol. 71, p. 481.

(13) " The Lancet : " 6th February 1904, p. 349.

(14) " Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government of India : " 1903, No. 3.

(15) " *Loc. cit.* (*vide* reference No. 1)."

(16) " Proceedings of the Royal Society : " Vol. 72, p. 419.

(17) " Journal of Experimental Medicine : " Vol. VI, No. 3, March 17, 1902, p. 277. " Univ. of Penna, Medical Bulletin." Vol. XV, No. 9, November 1902, p. 345. "Univ. of Penna, Medical Bulletin." Vol. XVI, Nos. 5-6, July-August 1903, p. 163.

(18) " Berliner klinische Wochenschrift : " 1902, Nos. 38 and 39 ; 1903, Nos. 2-4.

(19) " Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government of India : " 1903, No. 4.

(20) "*Loc. cit.*"

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